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(54) Micro flow processor.

(57) This invention relates to a micro flow processor (MFP) capable of generating highly accurate and reproducible micro flows. It is compatible with any type of solvent delivery system (reciprocating pumps or syringe pumps) and converts flow rates from ml/min into µl/min. The design of the MFP allows for isocratic and gradient elutions with flow rates in the sub-micro and microliter range. It consists mainly of a micromixer (static or dynamic) and a microsplitter with fixed splitting ratios to guarantee highly reproducible flow rates. The minimized dead volume ensures microgradient elution with virtually no delay. A method for the construction of the MFP is provided.

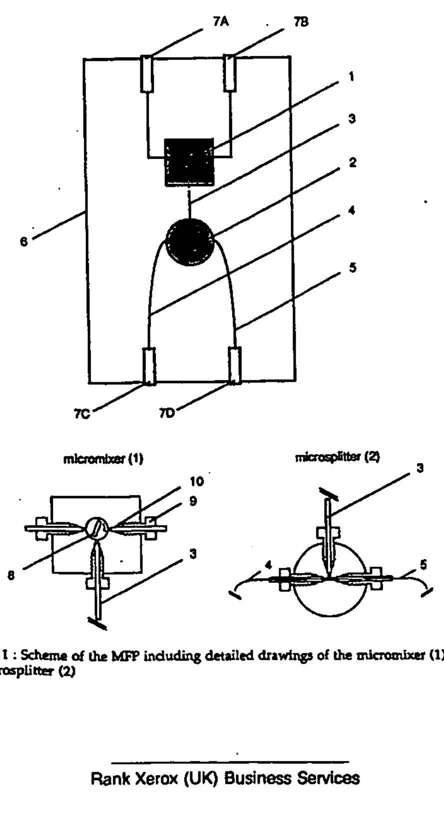

Fig. 1 : Scheme of the MFP including detailed drawings of the micromixer (1) and microsplitter (2)

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Field of the Invention

This invention refers to microseparation techniques in analytical chemistry such as micro liquid chromatography (MLC), capillary liquid chromatography (CLC), supercritcial fluid chromatography (SFC), open tubular liquid chromatography (OTLC), capillary electrophoresis (CE) and ancillary techniques.

Background of the Invention

The use of microseparation techniques such as MLC, CLC, SFC, OTLC, CE, etc. in analytical chemistry has attracted much attention, recently [1-4].
The increased separation efficiency, higher mass sensitivity, improved speed of analysis, and less solvent consumption are some of the advantages microseparation techniques can offer.
The analysis of biopolymers such as proteins, peptides, oligonucleotides, carbohydrates, etc., the trace analysis of contaminants such as pesticides, herbicides and fungicides etc., in ground water, soil, food and other matrices, require the highest degree of separation power and detection sensitivity. Microseparation techniques are often the sole alternative to fulfill these tasks. However, it has been reported by several authors [4-8] that one of the major limitation in microseparation techniques is the lack of reliable instrumentation. Although micropumps that allow gradient elution to be carried out are commercially available [9,10], the reproducibility and accuracy of gradient elution at low flow rates is not satisfactory [8]. Particularly when syringe pumps are used, the mixing of small flow rates of a few µl/min or even a fraction of these flow rates, which is necessary to run microgradients, is extremely difficult. The mixing chamber should have a volume in the sub-microliter range in order to minimize the dead volume (dwell-volume) and to reduce the delay time of the gradient formation. Further, the mixing device should provide an homogene mixing for any type of solvents and solvent composition used.
Mixing chambers which fulfill these requirements simply do not exist. An other drawback to using syringe pumps for the delivery of microgradients is the risk of microleakages. Small leakages are nearly impossible to detect because the resulting nanoliter volumes are not detected by the naked eye and due to their immediate evaporation. Furthermore, the flow equilibration of syringe pumps is rather time consuming and strongly affected by the solvent's compressibility and variations in temperature.
Facing all these difficulties, one may question if it is worthwhile to use syringe pumps even when their major advantage - the near pulse-free delivery - is required for flow-sensitive detectors (e.g., flame based, electrochemical, MS, etc.). With the advent of almost pulse-free reciprocating pumps, the conversion of milliliter flow rates into micro or even submicroliter flow rates using split flow techniques is an attractive alternative to deliver microflows. Reproducible gradient elution, however, is only possible when the changes in viscosity and compressibility are sufficiently compensated.
We have invented a method for the manufacture of a microflow processor (MFP), utilizing flow splitting techniques which consider the changes in viscosity and compressibility during the gradient elution. Such an MFP has the advantage of deliver highly reproducible and accurate microflows under both, isocratic and gradient conditions. Further, it allows for upgrading conventional pumps, including syringe pumps, into highly reliable and precise micropumps avoiding the difficulties of mixing and proportioning microscale flow rates.

Detailed description of the invention

Figure 1 shows a scheme of the MFP. It consists of two parts mainly: a micromixer (1), either static or dynamic and a microsplitter (2) with different types of restrictors. Both parts are connected via a tubing (3) and mounted in a protective box (6) to get a stable configuration. With a total of 4 ports (7A-7D), the MFP can be easily interfaced between the pump(s) and the microseparation system.

By setting the microsplitter after the micromixer, allows the use of conventional flow rates (e.g. 0.2 - 1.0 ml/min) throughout the mixing device. Thus, the difficulties of mixing microflows are avoided and use of standard micromixers guaranteeing for homogene mixing with minimal delay volume are allowed. The higher flow rates also avoid the risk of solvent misproportioning which is a severe problem when microflows have to be delivered. The splitting ratio is accomplished by capillaries of different I.D. and lengths (4,5) by making use of the Hagen-Poiseuille relationship :

$$F_{cap} = (\pi \, \Delta p_{cap} r_{cap}^4)/(8 \, L_{cap} \, \eta) \qquad [1]$$

where F_{cap} is the flow rate of mobile phase through the capillary, Δp_{cap} is the pressure drop along the

capillary, r_{cap} and L_{cap} are the radius and length of the capillary, respectively, and η is the viscosity of the mobile phase.

Fixed splitting ratios (e.g., 1:70 for reciprocating pumps, 1:30 for syringe pumps) avoid the need of flow calibration and allow use of two restrictors (4,5) only to accommodate almost any type of microflow. The microflow rates can then be calculated according eqn. 2:

microflow rate (µl/min) = input flow rate (µl/min) / split ratio [2]

For example, with a programmed flow rate of 700 µl/min (input flow) and a fixed splitting ratio of 1:70, a microflow of 10 µl/min is delivered.
To guarantee reproducible solvent composition during the gradient elution, the volume of the two restrictors, $V_{cap(4)}$ and $V_{cap(5)}$, should be as close to each other in value with respect to the split ratio.

$V_{cap(4)} = V_{cap(5)} \times \text{split ratio}$ [3]

To compensate changes in viscosities and compressibilities during the gradient elution and to minimize pulsations and other possible erratic fluctuations caused by the pump(s), the microsplitter (i.e. the adjusted capillary restrictors) provides additionally a backpressure. This backpressure (Δp_{cap}) should be preferably higher than the maximal back pressure of the microcolumn ($\Delta p_{max\ col.}$).

$\Delta p_{cap} \geqq \Delta p_{max\ col.}$ [4]

Assuming that the maximum backpressure a microcolumn can generate under relevant chromatographic conditions is much smaller than Δp_{cap}, then the flow delivery is almost unaffected by the type and composition of mobile phase.
Thus, by using the two adjusted restrictors (4,5), the delivery of microgradients is possible within a broad range of solvents of different viscosity and compressibility and for a large variety of capillary columns of different backpressure.

Micromixer

The micromixer (1) consist of a static or dynamic mixer with a small volume to minimize the delay volume. The volume of the mixer should be ≤ 500 ul. The static mixer can be made by using any type of conjunction (T-piece, Y-piece, union, etc) that combines the two flows and withstands pressures higher than 200 bar (e.g., stainless steel T-piece from Valco, Upchurch, Lee Viscojet micromixer etc). To enhance mixing and reduce delay volume, the conjunction can be packed with glass beads. Micromixers, e.g., rotating magnets (8), can be used as dynamic mixers. They can be made by machining a piece of stainless steel or titanium. Connections can be made with standard 1/16 or 1/32" high pressure fittings using standard nuts (9) and ferrules (10) or by soldering or gluing the tubings directly into the mixer.

Microsplitter

Basically, the microsplitter (2) consists of a same type of conjunction as the static micromixer but mounted in opposite direction in order to divide the flow into two streams. The volume of the splitter must be as small as possible. Therefore, the conjunction should have bores of I.D. ≤ 250 µm. (e.g. 0.25 mm I.D. T-piece from Valco, Upchurch, etc.).

Connecting tubing

The connecting line (3) between the micromixer and microsplitter can be made using any type of tubing with small I.D. and sufficient pressure resistance, e.g., stainless steel, PEEK, glass lined tubing, fused silica tubing, Polysil tubing, etc., Using static mixers, the connecting tubing further enables mixing of the fluids.

Restrictors and split ratios

The restrictors (4,5) are connected directly into the microsplitter, see Figure 1. They can consist of the same type of materials as mentioned under (3). Fused silica, however, is preferred, due to its availability in

different I.D. and ease of cutting in different lengths, pressure stability and inertness. The connection can be made with any type of fitting that withstands high pressures and provides sufficient chemical stability, e.g., 1/16 or 1/32" standard fittings made by a stainless steel nut and a Vespel ferrule. PEEK tubings or other polymeric materials of appropriate dimensions can also be used in combination with a nut (9) and ferrule (10). Alternatively, the capillaries can be glued (e.g. epoxy resin) into the microsplitter. The split ratio is fixed to ca. 1:70 for reciprocating pumps and ca. 1:30 for syringe pumps. Any other split ratio can be accommodated by selecting the I.D. and length of the capillaries according equation 1. In Table I the used restrictors are specified.

Table I : Restrictor specifications

split ratio 1:30

capillary	restrictor (4)	restrictor (5)
I.D. (μm)	15 ± 3	30 ± 3
length (cm)	20 ± 5	30 ± 5

split ratio 1:70

capillary	restrictor (4)	restrictor (5)
I.D. (μm)	15 ± 3	50 ± 3
length (cm)	20 ± 5	50 ± 5

Whereby, restrictors (4) and (5) connect the microsplitter with ports (7C) and (7D), respectively. The microflow is generated by restrictor (4), meanwhile restrictor (5) provides the necessary backpressure according equation [4].

ports and box

Standard 1/16 or 1/32" unions (e.g., stainless steel, Valco, Upchurch, etc.) are used for port 7A, 7B, and 7D.
To prevent dead volumes, port 7C is made with a through type union (e.g. Valco ZU-TM) which permits the tubings to be butted directly together.
The ports are fixed in the wall of the protective box and allow for easy interfacing between the pump(s) and the microsystem with standard 1/16" or 1/32" fittings.

Example

The installation of the MFP for isocratic and gradient operations is shown in Figure 2 A and B, respectively.

For isocratic separations (scheme A) the pump is connected via port 7A to the MFP, meanwhile port 7B is sealed with a plug (e.g. 1/16" Valco). The microsystem is connected to the MFP via port 7C by using a low dead volume tubing (e.g. fused silica ≤ 50 μm I.D., maximal length 20 cm, or polysil tubing from SGE, etc.).
Figure 3 illustrates the isocratic separation of polyaromatic hydrocarbons (PAHs) under Capillary LC

conditions, using a conventional reciprocating pump (e.g Kontron, Waters, HP, etc.) and the MFP. With an input flow of 200 µl/min and a a split ratio of ca. 1: 70 the flow rate of the pump was converted into a microflow of 3 µl/min.

In Table II the reproducibilities are summarized. With coefficient of variation of < 0.2 % in retention time, excellent reproducibilities have been observed.

Table II

Reproducibility under isocratic conditions (n = 12)						
n	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
1	5.524	7.423	9.008	10.033	11.894	14.036
2	5.539	7.449	9.039	10.069	11.941	14.093
3	5.543	7.454	9.051	10.081	11.956	14.108
4	5.554	7.466	9.059	10.094	11.968	14.121
5	5.546	7.463	9.057	10.089	11.964	14.124
6	5.546	7.463	9.063	10.093	11.978	14.129
7	5.548	7.466	9.059	10.094	11.978	14.139
8	5.562	7.477	9.067	10.092	11.940	14.083
9	5.545	7.455	9.045	10.075	11.950	14.093
10	5.554	7.461	9.053	10.084	11.959	14.111
11	5.543	7.454	9.043	10.073	11.948	14.099
12	5.539	7.454	9.049	10.084	11.954	14.111
mean	5.545	7.457	9.049	10.080	11.953	14.104
SD	0.009	0.013	0.016	0.017	0.023	0.027
CV %	0.170	0.176	0.171	0.170	0.187	0.191

For gradient elution (Figure 2B), using high pressure mixing, the two pumps can be connected via port 7A and 7B, respectively. For gradient delivery systems with low pressure mixing the installation is similar to the one described in Figure 2A. The use of the MFP for generating highly reproducible microgradients is depicted in Figure 4. A step gradient was programmed to measure the accuracy of the proportioning and effectiveness of mixing. Pump A was delivering pure methanol, and pump B methanol spiked with 0.2 % of acetone (input flow 350 µl/min, flow after split ca. 5 µl); UV detection at 254 nm. Excellent agreement between the programmed and measured step gradient was found. The delay volume (dwell volume) for the complete MFP (including mixer, connecting tubing, splitter, restrictor, port) is ≤ 5 µl.

Figure 5 shows the separation of digested β-lactoglobulin A under Capillary LC conditions. The retention time reproducibility for peptide A, B and C measured with consecutive injections over a period of several hours is listed in Table III. Without any thermostating, the CV values of ≤ 1.38 % for the tryptic peptides demonstrate the performance of the micromixer and the delivery of highly reproducible micrographients using the MFP.

Table III

Reproducibility of the microgradients (n=10)

n	Peak A	Peak B	Peak C
1	37.20	42.37	51.67
2	37.30	42.60	52.19
3	37.59	42.92	52.34
4	37.39	42.58	52.12
5	38.60	43.22	51.69
6	37.84	43.94	52.50
7	38.52	43.00	52.29
8	38.46	43.75	52.60
9	37.92	43.62	52.14
10	37.63	42.81	51.79
mean	37.85	43.08	52.13
SD	0.52	0.54	0.32
CV %	1.38	1.25	0.62

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Claims

1. A method of making a micro flow processor for the conversion of conventional flow rates (ml/min) into microflow rates (µl/min) in microseparation techniques using isocratic or gradient elution, which consists of the following steps:
placing the splitting device after the mixing device, so that the dead volume of said mixing device can be neglected by making use of higher flow rates resulting in improved mixing and proportioning of the delivered solvents;
connecting said mixing device with said splitting device by means of a connecting tubing;
inserting the restrictors directly into said splitting device to minimize dead volumes and to accomplish split ratios;
placing a restrictor in said splitter to compensate changes in viscosity, column backpressures and fluctuations of the delivery system; thereby the volume of the restrictor providing the backpressure is similar to the volume of the restrictor delivering the microflow with respect to the splitting ratio;

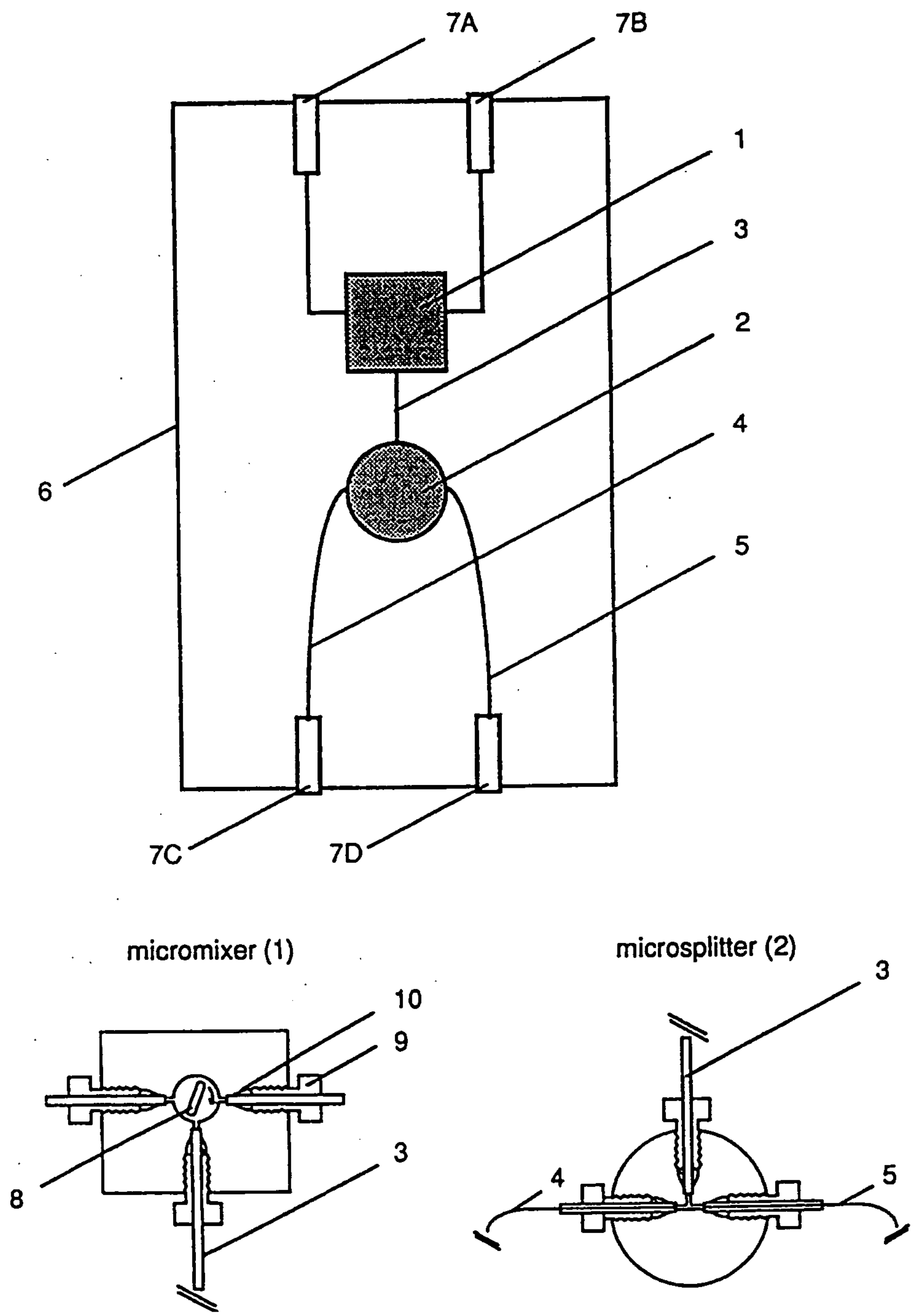

Fig. 1 : Scheme of the MFP including detailed drawings of the micromixer (1) and microsplitter (2)

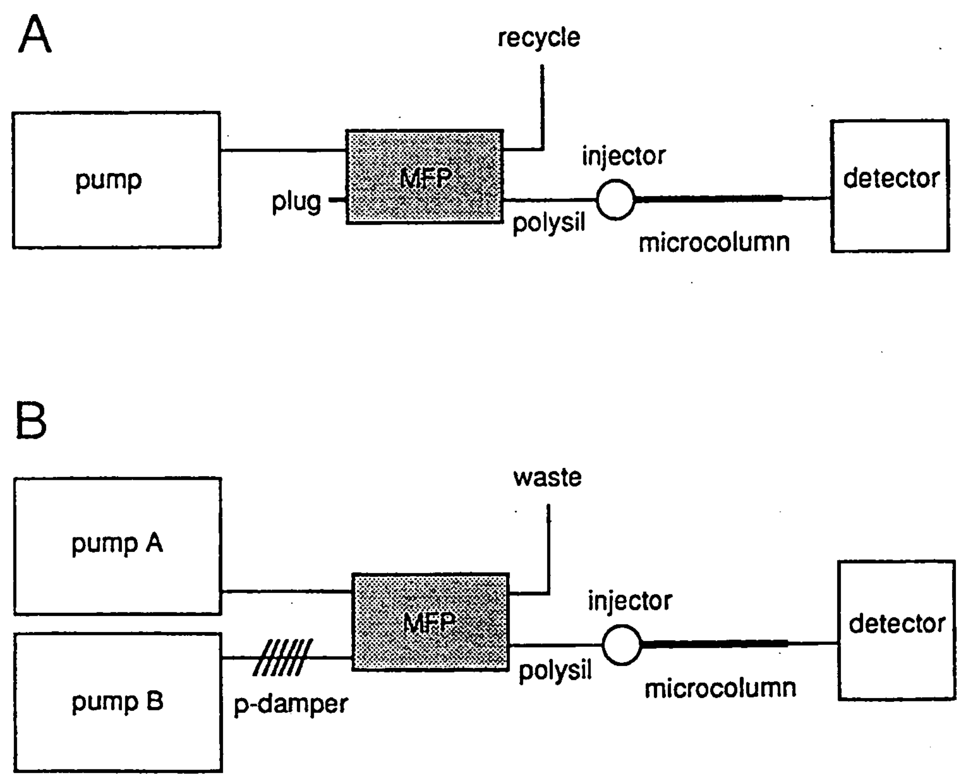

Fig. 2 : Instrumental set-up for isocratic (A) and gradient (B) operations

column:	Fusica™ 320 µm I.D. x 15 cm, C 18, 3 µm (LC Packings)
mobile phase:	acetonitrile/water (7:3)
pump:	conventional HPLC pump
mixer-splitter:	Acurate™ splitting ratio 1:70
flow after split:	3 µl/min
sample:	1. benzene 2. naphthalene 3. biphenyl 4. fluorene 5. anthracene 6. fluoranthene
injection:	60 nl
detection:	UV 254 nm Z-shaped flow cell

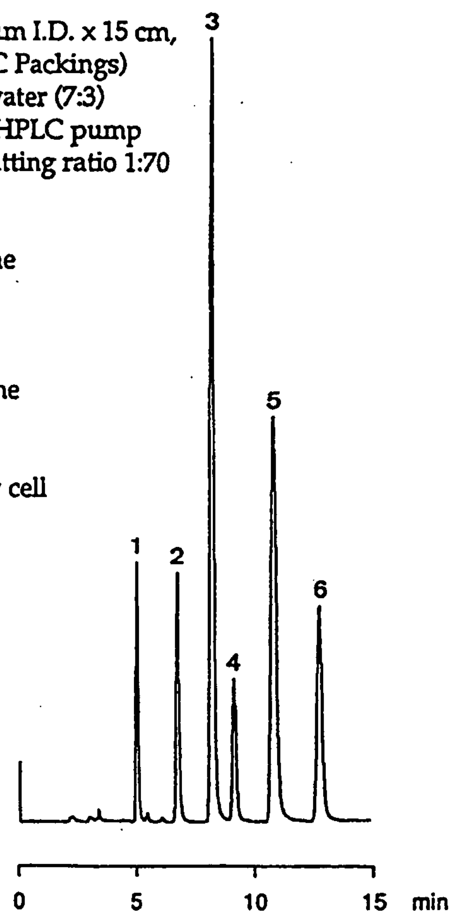

Fig. 3 : Capillary LC of PAHs using the MFP

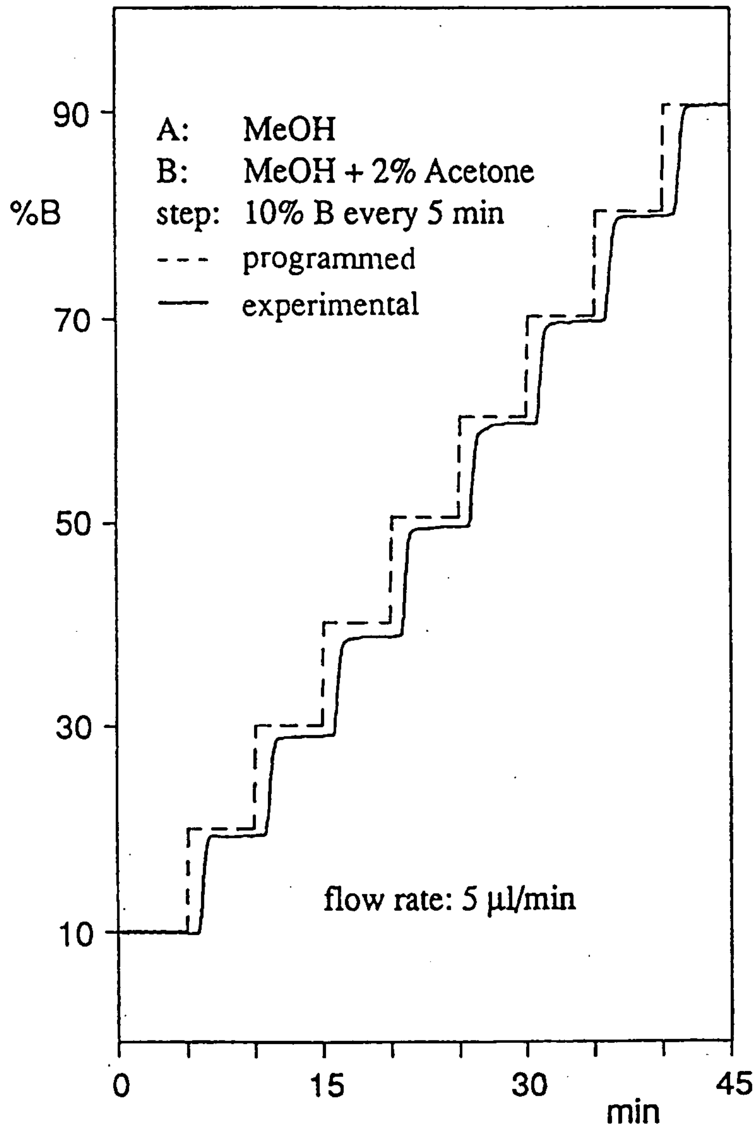

Fig. 4 : Step gradient using the MFP

column:	Fusica™ 320 µm I.D. x 15 cm, C 18, 3 µm (LC Packings)
mobile phase:	A) 0.1% TFA in water B) 0.08% TFA in acetonitrile water (8:2)
gradient:	5-50% B in 35 minutes
mixer-splitter:	Acurate™ splitting ratio 1:70 (LC Packings)
flow after split:	5 µl/min
sample:	tryptic digest of β-lactoglobulin A
injection:	100 µl (6.6 pmol)
detection:	UV 218 nm, 20 mm Z-cell (Kontron)

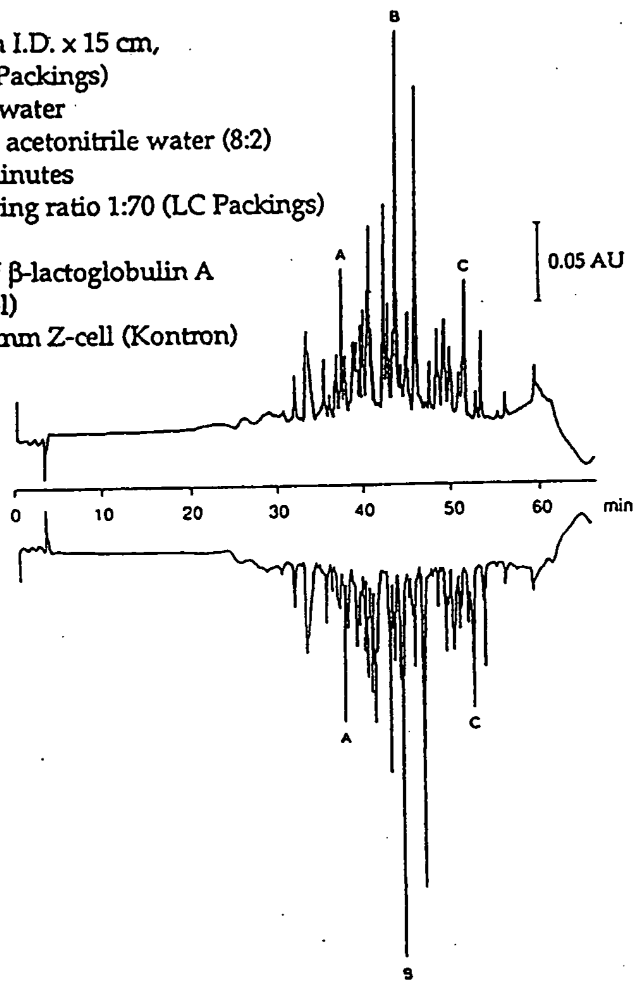

Fig. 5 : Tryptic digest of β-lactoglobulin A, using the MFP for the delivery of the microgradient (upper and lower trace are consecutive injections to illustrate the reproducibility

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EUROPEAN SEARCH REPORT

Application Number

EP 91 20 0080

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	ANALYTICAL CHEMISTRY. vol. 58, no. 7, June 1986, COLUMBUS US pages 1368 - 1372; SCHACHTERLE ET AL.: 'preformed gradient technique for microbore high-performance liquid chromatography ' * page 1368, column 1, paragraph 1; figure 1 *	1	G01N30/34
A	US-A-4 035 168 (JENNINGS) * column 7, line 18 - line 68; figure 1 *	1	
A	NL-A-7 801 663 (TRACOR) * page 7, line 19 - page 8, line 33; figure 3 *	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	27 SEPTEMBER 1991	ZINNGREBE U.

CATEGORY OF CITED DOCUMENTS

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